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Chemical and Physical Properties of Pectin

Pectin is a group of complex structural polysaccharides present in the cell walls of higher plants. Recent findings that pectin may act as a plant hormone, and that it is an important food fiber that may offer health benefits to humans, have heightened interest in its physical and chemical properties. Knowledge concerning the chemical composition and methods of pectin analysis is well advanced. Information on the primary structure of pectin and its chemical reactions has progressed significantly, but much remains to be learned. Least is known concerning the higher orders of pectin structure and how these are related to its properties as a plant component, a nutrient, and a texturizing agent in foods.

BACKGROUND

Pectin is a class of complex polysaccharides found in cell walls of higher plants (1). It is also an important food fiber (2) and a significant contributor to the texture of fruits, vegetables, and their processed products (3). Recent evidence shows that dietary pectin reduces glucose intolerance in diabetics and lowers blood cholesterol (2). Other evidence indicates that fragments of cell wall pectin act as elicitors of biochemical reactions in the defense of plants against attack by pathogens (4), and such fragments possibly induce metabolic processes important in fruit senescence (5). These results have encouraged basic studies to elucidate the chemical and physical properties of pectin. More precise knowledge of the chemical and physical behavior of pectin would supply base line information necessary to delay heart failure and reduce the incidence of certain cancers through proper nutrition; and it would provide for the development of more disease-resistant plants whose edible products taste better, would be less susceptible to post-harvest deterioration, and would be more readily processed.

CURRENT STATUS

Isolation and Purification

The method of isolation and purification should depend on the end use of the pectin. Commercially, apple or citrus peels are extracted with the goal of producing food grade pectin for gels. Typically, pectin is extracted with acid (pH 1.5 to 3) to inactivate endogeneous degradative enzymes, to prevent ester saponification, and to prevent alkaline degradation through beta elimination of uronide chains. The extracts are filtered or centrifuged to remove the insoluble residue. Next, pectin is precipitated from the acidic medium with alcohol or inorganic compounds such as aluminum or copper salts. These salts are removed from the precipitate by appropriate washing and drying. Often, during this process controlled de-esterification is achieved to obtain low methoxy pectins (6).

For research purposes, extraction and purification methodology has focused on obtaining pectin with maximum retention of the properties and structure that it possesses in the plant cell wall. These procedures provide valuable information on the role of pectin in the development and senescence of plant cell walls. Newer methods involve sequential extractions under mild conditions. Typically, plant tissue is extracted sequentially with aqueous alcohol, dimethyl sulfoxide, chelating agent, and mild base. In one such procedure (7), the sample is never completely dehydrated, to avoid fragmentation. Recent attempts to fractionate pectin have focused mainly on reducing size heterogeneity by gel filtration chromatography, and on reducing chemical heterogeneity by ion exchange chromatography, which separates according to degree of methyl esterification (DM) (8). Both of these methods have succeeded only partially.

Chemical Composition and Structure

Pectin is mainly a helical block copolymer of D-galacturonic acid and its methyl ester (9). These co-monomers are (1→4)-O-linked as poly(α-D-galactopyranosyluronic acid) in the backbone of pectin. Blocks are interrupted by (1→2)-linked α-L-rhamnopyranosyl inserts. Neutral sugars other than rhamnose found in pectin include galactose, arabinose, glucose, mannose, and xylose (10). At least three of these neutral sugars—arabinose, galactose, and xylose—have been found in pectin as short side chains, which themselves may be branched (11). The distribution of rhamnose inserts and neutral sugar side chains is not known with certainty. The partial

pectin structure in Fig. 1 incorporates some of these features. Generally, it is agreed that neutral sugar side chains are linked to rhamnose inserts in the backbone, but it also appears that linkages to oxygen in the galacturonate backbone exist. Recent work indicates that side chains in apple pectin are unequally distributed (12); some portions of the backbone have a high density of side chains and are designated "hairy regions," while other portions of the pectin backbone are completely devoid of side chains and are labeled "smooth regions." Degree of methyl esterification (DM) and neutral sugar content vary with method of extraction and plant tissue type. Table 1 lists the compositions of sugars found in low methoxy pectin from citrus peels (10). Acetyl and feruloyl ester groups have been found in pectin from a few sources, such as sugar beets. The acetyl esters are believed to be linked to galacturonate residues in the backbone, whereas the feruloyl esters are thought to be linked to neutral sugar side chains (9).

Methods of Analysis

The composition complexity of pectin has resulted in a broad spectrum of methods for its analysis. Some of the more popular or promising methods will be highlighted here. Of the several colorimetric methods developed to analyze for galacturonic acid content, reaction of pectin with m-hydroxybiphenyl in heated acid media to form a colored product appears to have gained most favor (13,14). There are numerous methods to determine degree of methyl esterification, including base titration before and after saponification, colorimetric determination of saponified methanol as quantitatively oxidized formaldehyde, and determination of carboxyl and methyl ester groups by infrared spectroscopy in deuterated water (15). The infrared method requires calibration against known standards, whereas differential titration also yields the content of nonmethylated galacturonate residues. Determination of neutral sugars by gas chromatography, after hydrolysis and conversion to volatile derivatives, appears to be the method of choice. Although several derivatives and stationary phases have been tried, alditolacetates and high performance capillary columns appear to be gaining favor. Content of acetyl and feruloyl esters are analyzed after base hydrolysis. Acetyl esters produce acetic acid, which is separated by distillation and determined by titration. Feruloyl esters are converted to ferulic acid, which is analyzed by the Folin-Ciocalteu phenol reagent.

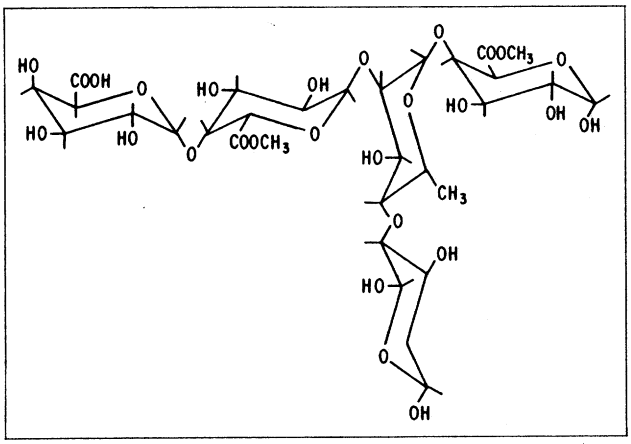

Figure 1 Portion of a pectin molecule.

Table 1 Percentages by weight of sugars in citrus pectin*

Sugar	Percentage total
Galacturonate	78.0
Galactose	8.49
Rhamnose	7.00
Arabinose	2.03
Glucose	2.03
Mannose	1.32
Xylose	1.12

* Percentage of esterified galacturonates is 37%. From reference 10.

Characterization of Molecular Size Parameters

Pectin has been characterized by virtually every significant method applicable to macromolecules. In spite of repeated characterizations from a variety of plant tissues, controversy remains regarding the molecular size parameters of pectin. Much of the controversy arises from the tendency of pectin to aggregate, disaggregate, and fragment according to the method of sample preparation, the conditions, and the kind of molecular weight measurement. No single molecular weight or unique molecular weight distribution has been established for pectin. Pectins are obtained as solubilized fragments from a complex plant cell wall. Apparently, molecular weight distributions depend on the variety and maturity of plant, as well as on the method of extraction. Furthermore, because distributions are polydisperse, comparison of molecular weights is possible only for those techniques that give the same average. Comparison of number average molecular weights (M_n) from end group analysis, high performance size exclusion chromatography (a form of gel filtration chromatography), and osmometry revealed that commercial citrus pectin undergoes concentration-dependent disaggregation (16,17). Furthermore, this pectin was asymmetric in shape and underwent changes in size with changes in the ionic strength of the solvent. Size changes with changes in ionic strength are consistent with the polyelectrolyte nature of pectin. High methoxy citrus pectin was found to have aggregated M_n values of about 40 000 and disaggregated values of about 15 000.

Characterization by dynamic and static light scattering of mildly and sequentially extracted pectin from apples revealed that two kinds of pectin were extracted (7). Pectin attributed to the middle lamella of a cell wall had a weight average molecular weight (M_w) of 4.2×10^6, a broad molecular weight distribution, and a shape best approximated by a stiff, non-free draining coil. Pectin attributed to the primary cell wall had an M_w of 1.1×10^6, and a shape best approximated by a sphere of inhomogeneous density. In contrast to previous results, these pectins did not exhibit the typical nonlinear change in molecular size when excess scattered light intensity was measured as a function of concentration in pure water. Perhaps it can be concluded from these results that large, intact pectinaceous sections of the cell wall had been solubilized for the first time. It should be noted that in pure water, commercial citrus pectins appear to aggregate to sizes comparable to those measured for mildly extracted pectins (18).

Binding Properties

Many of pectin's unique properties can be attributed to its ability to interact with itself and with cosolutes. In gels, pectin self-aggregates or interacts with cosolutes to

form a three-dimensional network capable of entrapping water. High methoxy pectin (HMP, DM > 70%) or medium methoxy pectin (DM about 57 to 60%) gels under acid conditions in the presence of sugars (65% solids). Sugars may promote aggregation by removing bound water from HMP, reducing ionic repulsions, and stiffening the polymer chains (19). At constant pH, HMP gel strength and rate of gelling increase with DM. As the DM decreases, the pH must also decrease to induce gelation. Low methoxy pectins (LMP, DM < 50%) gel in the presence of calcium or other divalent cations (9). For LMP, calcium promotes gelation through interchain binding. LMP gels exhibit higher gelling temperatures and greater gel strength with decreasing DM.

Increasing molecular weight increases the gel strength of HMP and LMP, possibly by promoting cooperative self-interactions. Circular dichroism provides direct spectroscopic evidence that divalent cations promote gelation through interchain bridging of carboxyl groups (20). Clinical studies suggest that pectin lowers blood cholesterol, one possible mechanism of which involves the binding of the bile acids that are metabolic products of cholesterol. In support of this hypothesis, recent evidence indicates that pectin may bind bile acids through calcium bridges between the carboxyl groups of pectin and bile acid molecules (21).

Pectin binds monovalent cations in addition to divalent cations. In a study with a series of sodium pectinates of differing degrees of methyl esterification, it was shown that the extent of counter-ion binding increased with charge density provided by carboxylate groups along the pectin chain. Furthermore, reducing pectin aggregation reduced counter-ion binding as well (17). Electron spin resonance studies (22) of divalent cation binding to pectin in apple cell walls supported the concept that divalent cations form interchain bridges between adjacent "smooth" regions of pectin molecules ("egg box model") and that binding is a sequential cooperative process. In the case of Cu^{2+} the nearest neighbor approach was 12 Å whereas it was 14 Å for Mn^{2+}.

Chemical Reactions

The reactions of pectin are those characteristic of polysaccharides, esters, and organic acids (9). Pectin that contains o-feruloyl and/or o-acetyl ester groups reacts in a fashion that is characteristic of these functional groups. The discussion in this section will be confined to reactions that have special significance in relation to pectin.

Pectin undergoes acid-, base- and enzyme-catalyzed depolymerizations. Hydrolysis of all glycosidic bonds except (1→4) self-linked α-D-galacturonic acids occur preferentially at acid pH. Thus pectins often are extracted from plant matrices by controlled acid hydrolysis. Furthermore, very pure polygalacturonic acid (ca. DP 25) can be prepared by harsher conditions of acid hydrolysis. Base-catalyzed depolymerizations occur at neutral and higher pH's. These are beta elimination reactions involving concurrent endo-depolymerization, deesterification, and double bond formation. Conditions favoring beta elimination reactions are similar to those promoting dissociation of noncovalent hydrogen bonds. Thus, it is often difficult to distinguish between decreases in molecular weight of pectin induced by the dissociation of hydrogen bonds and those induced by the beta elimination reaction. The relatively high susceptibility to enzymatic and acid-catalyzed hydrolysis of the neutral sugar sidechains in pectin may be important in certain metabolic processes occurring in the plant. For example, evidence suggests that the galactose-containing fragments from pectin may elicit ethylene production in plants, which is important in the process of senescence (5). Endogenous plant polygalacturonases that depolymerize pectin randomly (endo) and at chain ends (exo) have been identified (23). All polygalacturonases depolymerize deesterified pectins, exclusively. In certain fruits, polygalacturonases have been associated with the ripening process. Interestingly, the endo-uronases have been associated with rapid ripening whereas slow ripening occurs when exo-uronases are present.

Pectin lyase depolymerizes by trans-elimination of the proton on carbon 5 and the glycosidic oxygen of galacturonate, whereas polygalacturonase merely cleaves the glycosidic oxygen. Although polygalacturonase, pectin lyase, and beta elimination reactions all cleave galacturonate, lyase usually requires an ester group and beta elimination always requires an ester group; both produce an unsaturated end group. Lyases are not generally endogeneous to plants, but are produced by microorganisms. Pectin methyl esterase activity has been found in plant cell walls, but esterase activity has not been correlated with softening. This is a rather surprising result, given the requirement that polygalacturonases are specific to deesterified pectins. Reactions specific to the reducing sugar end group are important in that they permit determination of number average molecular weights and provide a method of following the course of depolymerization reactions. Chlorite oxidation of end groups has been used to determine pectin molecular weights (24), whereas several color reactions specific for reducing sugars have been used to assay galacturonic acid (23). Reagents such as ammonia and amines will convert ester groups to amides. Ammonia partially amidates and hydrolyses pectin ester groups. Amidated low methoxy pectin gels more readily than corresponding nonamidated low methoxy pectin (9). Recently, pectin from beets has been gelled by intermolecular, oxidative coupling of feruloyl ester groups through their aromatic rings. Coupling was achieved with a mixture of hydrogen peroxide and peroxidase (25). Low molecular weight and the presence of acetyl groups normally prevent beet pectin from gelling.

FUTURE DIRECTIONS

It appears that the structure of pectins can be categorized by level of organization in a manner analogous to the categorization of the structure of proteins. As the various levels of pectin organization become better understood, this knowledge will be applied to understand how pectin functions in the plant, as a functional agent in foods, and as a nutrient. With respect to the primary (covalently bonded) structure, the placement of side chains and the sequence of neutral sugars within them is likely to be investigated, as is the placement and sequence of rhamnose and ester groups in the backbone. Secondary and tertiary structure probably will be investigated by attempting to understand the role of intramolecular interactions on the size, shape, and stability of pectin. Questions may be answered about the interplay of ionic charge density with intramolecular hydrogen bonding and the effect of hydrophobic interactions on the secondary structure of pectin. Aggregation could be studied further to yield information on the quaternary structure

as determined by the intermolecular interactions of pectin. The relative roles of nonbonded (ionic, hydrogen, and hydrophobic) interactions in holding pectin aggregates together is likely to be investigated. Furthermore, investigators will attempt to ascertain whether covalent bonds hold pectin aggregates together. Another question to be answered concerns the extent to which pectin aggregates are metastable in nature, rather than assuming stable equilibrium states. Other investigations of quaternary structure will involve the ability of pectin to preferentially bind cosolutes in an aqueous environment. A major question to be investigated concerns the fragmentation of pectin, and to what extent this involves the breaking of covalent bonds compared to the dissociation of physically adsorbed fragments.

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GLOSSARY

circular dichroism spectroscopy: Spectra produced by the differential absorption of circularly polarized light in directions parallel and perpendicular to the long dimension of an asymmetric molecule.

dynamic light scattering: Technique for irradiating a molecule with visible light and measuring the time-dependent intensity of the light reflected from the molecule to obtain information on rotational motion, size, shape, and molecular weight of macromolecules.

electron spin resonance: Effect produced by absorption of the specific frequency of radiation required to flip the angle of an electron's spin.

endo-pectin lyase (E.C. 4.2.2.3): Enzyme that catalyzes the random depolymerization of esterified galacturonate residues in the pectin backbone through a trans-elimination reaction at the C-5 carbon and the glycosidic oxygen.

endo-polygalacturonase (E.C. 3.2.1.15): Enzyme that catalyzes the random depolymerization of nonesterified galacturonate residues in the pectin backbone through hydrolysis of the glycosidic oxygen.

food fiber: Those structural components of plants that can be ingested but are largely indigestible by humans.

glycosidic bonds: Covalent bonds joining adjacent sugar residues through an oxygen atom common to both residues.

high performance size exclusion chromatography: Technique in which a mixture of molecules is separated and analyzed according to their molecular sizes by forcing the dissolved molecules through a column packed with porous beads whose average diameter is less than 15 μm and whose distribution of pore sizes corresponds to the distribution of diameters of the analyte.

middle lamella: Intercellular region of the cell wall.

number average molecular weight: Average obtained by measuring colligative properties, by end group analysis, or by determining an entire distribution of molecular weights for a macromolecule that is polydisperse with respect to molecular weight.

pectic substances: A group of closely associated complex polysaccharides in which the major component is 1–4-linked α-D-galacturonan, and which are found in the primary cell wall or the middle lamella of higher plants.

weight average molecular weight: Average obtained by measuring excess scattered light, distribution in a centrifugal field, or the entire distribution of molecular weights for a macromolecule that is polydisperse with respect to molecular weight.

static light scattering: Technique for irradiating a macromolecule with visible light and measuring the time-independent excess light intensity reflected from the macromolecule, in order to obtain the size and weight average molecular weight of the molecule.

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